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# Further study on *Sphenophrya dreissenae* Dobrzańska 1958 *(Ciliata, Thigmotricha)*

## Dalsze badania nad *Sphenophrya dreissenae* Dobrzańska 1958 *(Ciliata, Thigmotricha)*

In a previous paper (Dobrzańska 1958) the description was given of a new species *Sphenophrya dreissenae* Dobrz. from the gill epithelium of the *Dreissena polymorpha* Pall. General morphology was given as well as the morphogenetic changes occurring during gemmation and transformation of the bud into the mature stage and the silver absorbing system of this ciliate. The present paper gives new data concerning the structures of the particular organella, the nuclear transformations during fission, the conjugation as well as comparative studies so as to establish the systematic situation of the ciliate studied and the relations within the family of *Sphenophryidae*.

## Material and methods

The *Sphenophrya dreissenae* Dobrz. material was taken from *Dreissena polymorpha* from the littoral of the Czerniaków Lake in Warszawa. Up to 100 per cent of the molluscs were invaded by the protozoa in particular colonies. This usually coincided with a considerable intensity of invasion. The invasion diminished markedly in the molluscs scattered on the sublittoral area. *Sphenophrya dreissenae* Dobrz. was often found to accompany *Hypocomagalma dreissenae* Jarocki et Raabe and *Conchophthirus acuminatus* Clap. Lachm. No direct relation was found between the occurrence of these protozoa. However, there were marked seasonal changes in invasion intensity. Marked peaks of invasion intensity could be observed twice a year, namely in the spring and during the autumn. The months when the peaks occurred varied with the meteorological conditions. In most cases, however, the peak occurred in late April and in mid October. The above data are casual; they cover a three year period in which only the winter time — when the reservoir was frozen — is not included.

*Sphenophrya dreissenae* Dobrz. was confirmed in the molluscs from other reservoirs of Poland too. The present authoress confirmed its occurrence (although less significant) on the harbor piles of the Hydrobiological Station

of the Polish Academy of Sciences on the Mikołajki Lake in the *Dreissena* colonies and in the material from the Żarnowieckie Lake littoral (Pomerania) which was collected by A. Kaczanowski and prepared by Z. Raabe. *Sphenophrya dreissenae* was also confirmed in the molluscs from the rivulet Piaśnica, Pomerania, and, sporadically, in those from the mouth of the rivulet Płutnica near Puck, Poland. The sporadical occurrence may be caused by the host being not numerous and sparsely scattered as a result of the rivulet mouth being salty. *S. dreissenae* Dobrz. seems to be a common parasite in both running and stagnant water.

The present authoress found the intensity of the *S. dreissenae* invasion to be higher in the young *Dreissena* specimens studied. This fact is remarkable because of Miassnikowa's 1930 confirmation of *Sphenophrya sphaerii* occurrence in young specimens of *Sphaerium corneum* L., and Polianskij's 1951 description of that of *Sphenophrya minor* in young specimens of *Cardium*. Similarly, J. et Z. Raabe who had studied *Urceolariidae* (1960), found *Sphenophrya dosiniae* Ch. Lw. invasion to be much higher in the young *Mya arenaria* L. specimens than in the grown-up ones.

The material collected by herself , the present authoress had studied in vivo in smears applying the phase contrast on some occasions. Besides the study was carried out on preparations executed by methods as given below:

| Fixation | Staining |
|---|---|
| infravital | neutral red<br>Janus green B |
| methyl alcohol | Giemsa after Romanowsky |
| Schaudinn | iron haematoxylin after Heidenhain (I.H.)<br>Feulgen after Pearse<br>Mallory after Cassom |
| Carnoy celloidin - | Feulgen after Pearse<br>gallocyanin<br>carmin after Best |
| celloidin - | Schiff and hyper-iod acid<br>pyronine and methyl green after Brachet |
| Champy, bichromate K | iron haematoxylin after Regaud<br>acid fuchsine after Altmann |
| Bouin | Sudan black |
| Zenker-formol | Sudan black |
| dry preparation | silver impregnation after Klein |

Parallel with *Sphenophrya dreissenae* Dobrz., the present authoress had also carried out comparative studies on *Sphenophrya sphaerii* Miassn. from *Sphaerium corneum* from the Żarnowieckie Lake and from the fresh water reservoirs near Warszawa ($AgNO_3$ impregnated preparations after B. Klein, stained with the Schiff reagent after Feulgen). Besides, the authoress had at her disposal the materials of Z. Raabe concerning *Sphenophrya dosiniae* Ch. Lw. from *Cardium edule* and *Mya arenaria* from the Gulf of Gdańsk and Puck in the South Baltic Sea ($AgNO_3$ and I. H. preparations).

## General body shape and external structures

The shape of adult form resembles an elongated helmet. The protozoans attach themselves to the ciliary epithelium of the gills by a process lying on the edge of the attachment surface corresponding to the „suçoir" or with the entire surface. This surface is a typical sole. On account of the elongation of the body, this sole takes the form of an elongated ellipse. In the description in connection with the torsion and other changes of the body axis, the phylogenetic orientation of the body will not be taken into account, only the morphologic orientation, considering the sole as the ventral surface of the body, the apex as the dorsal side and the „suçoir" as the anterior end. Thus two systems of rows of basal granules begin at the apex, which is generally slightly displaced towards the posterior end of the body. Both these systems run in clockwise direction. 4 rows (2 + 2) extend posteriorly from the apex reaching to the attachment surface. The second system of 5 parallel rows of basal granules also originated from the apex but more anteriorly in relation to the former system. The rows first run in a mild curve towards the sole almost parallelly to the other system; however, they subsequently bend up sharply forming a semicircle around the body of the protozoan and then descend towards the sole and disappear.

Characteristic of the adult trophic form is the clockwise spiralization of the surface structures of the body (looking from above, from the morphological side of the apex). The degree of the torsion may vary. The maximum values are as follows: approximately 90° for the four kinetes on the left (in accordance with the orientation of the basic corpuscules systems in *Sphenophryidae* Chatton et Lwoff 1950), and approximately 200° for the right system of five kinetes. The location of the apex can be easily made out (even when looking from above) owing to the meshes of the silver absorbing net forming a whirl around the apex (see phot. 4, 5). Polygonal meshes of the net spread uniformly over the whole body except the sole. The shape of the meshes reflects the changes going on in the cortical structures of the ciliate. The longitudinal axis of the individual polygonal mesh usually corresponds to the vector of growth of that particular portion of the cortical structures. This is in perfect agreement with Raabe's 1949 observations on the "dynamic net" in *Sphenophrya dosiniae*.

Erratic basal corpuscules are often observed in the region surrounding the sole. The former resemble the „granules erratiques" which Chatton et Lwoff 1921 have confirmed in *Sphenophrya dosiniae*. In most cases the net of the silver absorbing system and the kinetosomes disappear at the limit-

-line between the lateral surfaces (morphologically) and the sole of the ciliate according to Chatton, Lwoff et Lwoff 1931. The limit-line between the sole and the rest of the pellicle is marked very distinctly. The sole forms an elongated elliptic adhesion surface. Neither basal corpuscles nor the silver absorbing net system does occur here. Solely, irregular granules are present in the ectoplasm which becomes brown when silvering is being carried out. The character of the *Sphenophrya dreissenae* sole is in accordance with that of *Sphenophrya sphaerii* as described by Raabe 1949.

## Cytoplasm

The cytoplasm is colourless, transparent but differentiated: that zone of it which is adjacent to the sole is doubtlessly denser still very elastic. The ectoplasm adjoining the sole stains very intensively. Carbohydrates are present there as shown by the Schiff reagent and hyper-iodic acid reactions. Apparently this is the mucigenous zone too for this portion of the cytoplasm becomes intensely blue when stained after Mallory's method, mod. Casson. This could be explained considering the adhesive character of the surface. Besides the ectoplasm adjoining the sole will be more intensely stained with I. H. and becomes uniformly yellow when silvered which probably proves this zone density being higher. Similarly stains also the adhesive surface ectoplasm in *Pelecyophrya tapetis* Ch. Lw., *Sphenophrya dosiniae* Ch. Lw. and in *Sphenophrya sphaerii* Miassnikowa. There are no data concerning other species. The central part of the cell is occupied by the nuclear apparatus with the homogenous cytoplasm zone stretching next to it. This cytoplasm accounts for the halo which is observed both in living specimens and in stained preparations. Such a cytoplasm zone adjoining the nucleus has been often sketched by Chatton et Lwoff in their drawings representing *Pelecyophrya tapetis, Sphenophrya dosiniae* and others.

However, the character of the peripheral layer of the endoplasm is different. This is an alveolar zone containing at least two kinds of inclusions:

1. Vacuole varying in size and position which however at the trophic stages is usually situated in the region of the suçoir, (according to the terminoges is usually situated in the region of the suçoir (according to the terminology of Chatton et Lwoff). This vacuale stains with neutral red. Neither could be observed any excretory opening nor any rhythmical pulsation. It seems not impossible that it serves for osmoregulation, its dimensions being dependent on the slime density and on the osmotic liquid pressure in which the ciliate is being observed. After a few minutes immersion in tap water the vacuole will inflate which does not happen in the ciliate examined in the slime. All these observations are in accordance with those carried out on the C. V. in fresh and brackish water *Sphenophryidae*. In adult forms or at the division stage the ciliate may exhibit two vacuoles which push apart furing the cytoplasm movements at budding period. Buds with no vacuoles occur very often. The vacuole diameter ranges from 1.5 to 5 μ with 1.5 μ on the average in the bud and 2.5 μ in the adult specimen.

2. Small ectoplasm vacuoles, 4 to 14 in number. They are spherical in shape and all of them are about 1 μ in diameter. Some of them penetrate into the

bud during the division period; there is, however, no rigorous segregation of them. The number of the vacuoles is higher in the larger specimens. The distribution of the former is almost even. The phasic limits may be very easily observed under an ordinary microscope due to the vacuoles ability to refract light very readily. The vacuoles appear in the shape of light spots in the I. H. preparations and in those stained with light-green-tinted Feulgen. The present authoress failed to discolour them with gallocyanine or Sudan black B too. Neither their shape and disposition correspond to the mitochondria. As regards cytoplasmic inclusions, lipoidal grains were noted only by Kozloff in *Lwoffia cilifera* Kozl. As to other protozoa the precise data are lacking.

No constant particles nor any fragments of the host's cells were found by the present writer in the cytoplasm. Consequently, *Sphenophrya dreissenae* seems to feed osmotically only.

One or two formations may be seen in the cytoplasm of some *Sphenophrya dreissenae* specimens. These are flattened hyalinous ellipses with edges readily stained with I. H. Their appearance corresponds to the „petits cylindres sidérophiles" which Chatton et Lwoff 1922 discovered in *Pelecyophrya tapetis* or to the "rätselhafte Gebilde" in *Sphenophrya sphaerii*, described by Miassnikowa 1930 and Raabe 1938, the "baguettes tabulaires" in *Sphenophrya dosiniae* Chatton et Lwoff 1921, "les ébauches des suçoirs" in *Gargarius gargarius* Chatton et Lwoff 1934, Raabe 1935, and to the spherical "primordium of sucker" in *Lwoffia cilifera* described by Kozloff 1955. It is interesting to note that their structure resembles most closely the hypothetical one as described and sketched for this form by Raabe 1949.

The present authoress examined the mitochondria mainly in vivo using Janusgrün B or — in preparations — using acid fuchsine after Altmann or I. H. after Régaud. The results were obtained with the Janusgrün B method. The negative results obtained with other methods might be due to the dye penetrating with more difficulty into the total ciliate material than into slices.

Mitochondria occurred subpellicularly in the shape of small granules distinctly visible under hom. imm. × 90. They were arranged irregularly and formed bead-like agglomerations. In accordance with the findings of Fauré — Fremiet 1910 who studied *Trichodinopsis paradoxa* Clap. Lachm. the present writer found that mitochondria in *Sphenophrya dreissenae* were also proportionally larger than those of the molluscs gill cells. On the contrary, no relation was found between the basal corpuscles and the mitochondria which were confirmed in some *Holotricha* (Chatton et Brachon 1935).

## Nuclear apparatus

The nuclear apparatus of *Sphenophrya dreissenae* consists of one macronucleus (Ma) situated centrally in the cell, and of one micronucleus (Mi) which situation is variable in respect to the macronucleus.

The nuclear apparatus was examined using Feulgen reaction, the gallocyanine, and by staining with pyronine and methyl green. The dimensions of Ma were 9 — 13 μ × 5 — 10 μ in adult form, and 5 — 8.5 μ × 4 — 8 μ in buds.

Usually the trophic form was pyriform with its longer axis parallel to the sole surface. The Ma was, on the contrary, more spherical in shape at juvenile stages, with its longer axis being parallel to the long axis of the bud; thus, together with the change of the situation of the morphological axis a shifting of Ma occurred. Besides, there was also variability in the shape of Ma. This seemed to be closely related to the life cycle. At splitting stages (and fairly often at pre-division phase) Ma was becoming elongated assuming the shape of a stick or of a sausage, and even that of a shinbone, thin in the middle and disposed parallelly to the sole.

Macronucleus had its own distinct membrane which very markedly refracted light. This membrane was visible both in vivo as well as in the fixed preparations. The membrane was not uniform in structure: I. H. staining as well as that acc. to Brachet's method revealed the presence of an albumen substratum which is intensely stained with pyronin; the substratum was almost completely disguised with a significant number of granules containing DNA which stained readily with methyl green and Schiff's reagent. These granules could be seen flowing out of an injured Ma and at degeneration stages of the latter, e. g. at gradual fragmentation stage of the Ma following conjugation.

Micronucleus was spherical in shape, 3 — 3.4 μ in diameter, being thus rather large. This resembled conditions in other *Sphenophryidae,* e.g. the large Mi in *Gargarius gargarius* according to Chatton et Lwoff 1934. Mi was usually situated apically in mature form, while in juvenile form it was in the region of the sucker similarly as in other *Sphenophryidae.* Both Mi and Ma were in the zone of homogenous cytoplasm. Mi at rest stage exhibits no uniform structure. An endosome, rather large in size and less readily stained, could be seen inside the Mi surrounded by a layer that stained intensely with nuclear stains. The endosome seemed not to correspond to the nucleolus for it stained solely with pyronin (very badly) in contrast to the nucleoli of epiderm cells in the nearby smears of *Dreissena.* The occurrence of such a heterogeneous structure of Mi was observed in many ciliates, e.g. in *Bursaria* (Poljansky 1934). A reverse phenomenon was observed by Stephens 1901 in *Boveria subcylindrica* Issel in which the central part of cytoplasm was highly acid-absorbing and stained very readily in contrast to the alkali-absorbing parts of Mi. However, Stephens noted the two zones' identicity in I. H. staining. Structure of Mi in *Sphenophrya dreissenae* and the character of mitosis were also in accordance with Kidder's 1933 observations on the *Kidderia mytili* (De Morgan). Finally, this structure corresponded to the drawings of *Sphenophrya dosiniae* published by Chatton et Lwoff 1950 as well as to my own observations on Mi structure in *Sphenophrya sphaerii.*

## Budding

The rate of propagation seems to be variable and no twenty-four-hour rhythm of splitting was observed. However, there was a relative synchronization of splittings in individual populations from one mollusc specimen.

Both budding and conjugation had a mass character. Even not fully transformed forms could already attain the preliminary stage of budding. This

might signify either the presence of karyokinesis or the beginning of Ma disconnection, or the simultaneous occurrence of both of these phenomena; this, further, might signify that characteristic changes could occur in the cortical zone.

Bud formation was usually preceded by a preparatory stage at which changes in body proportions of the protozoon occurred. The sole length was proportional in relation to the body height in the trophic forms (according to the morphological orientation of the body — Dobrzańska 1958). The sole-length:body-height ratio representing the degree of sticking to the host epithelium was approximately 1 — 1.2. An elongated conical shape was characteristic of the pre-division stage with the sole-length:body-height ratio varying within the range 0.6 — 1.0.

The vectorial character of the height development caused the straightening of the upper portion of kineties in both systems (the despiralization). Such a trophont form transforming into tomont had a distinct break of kineties in the apical direction. However, the proportions of such tomont corresponded to those of the juvenile form, the tomite, i.e. they corresponded to the proportions of the bud before spiralization. The intensive directional body development was related with the meshes in the silver absorbing net being different in shape, and stretching along the growth vector. At the same time there occurred laceration of the kineties at the site of their breakage. Firstly five kineties of the right system were lacerated (n.b. there is no regular succession of kineties breakings in this system) and then only those of the left one. The pellicle indentation cutting off the bud began to form at this period. However, the dividing furrow did not only cut off the swelling bud, but also cut out the central part of cytoplasm which resulted in the conical sucker (suçoir) — that was being formed at that period — being sunk into the body of the maternal organism.

Here and there, cilia occurred during the period when the bud was being cut out; these cilia, however, disappeared when the bud had completely slipped out of the maternal body. They were 5 μ in length and disappeared as unnecessary and even harmful at the moment when the ciliate had stuck to the epithelium of the host. Their sporadical occurrence resembles the variations in cilia of *Pelecyophrya tapetis* reported by Chatton et Lwoff 1922. The mother specimen might become wrinckled during the period of the dividing furrow formation. This was indicative of a considerable degree of plasticity of the sole. The last connection spot between the specimens was the suçoir of the bud trough which cytoplasm was passing from the maternal body forming a somewhat lighter track. However, the present authoress could confirm no formation of trough. The repeatedly dividing specimen was asymmetric in shape having a shifted apex (Dobrzańska 1958). The bud was then being formed correspondingly on the side of the maternal specimen. The abnormal reciprocal arrangement of the specimens implied incomplete penetration of the kineties into the bud. Side by side with a full set of the left system solely three or four kineties of the right one occurred in such cases. The fact of this anomaly occurring very often could possibly be related to the absence of natural selection in respect to this character because of its being without any biological significance. It was, thus, merely a feature determining the range of individual variability.

The forming bud contained no part of the sole, under normal budding conditions. Thus, the sole formed absolutely de novo as a rule.

The nuclear changes related with the budding may proceed in different ways. Usually the dividing was initiated in the Mi with its dimensions increasing and its structure becoming apparent. At the prophase stage four chromatin thickenings were formed which were connected to each other by a fine network of chromatin strings covering the peripheric parts of the Mi. Next, four string-like bands differentiated which stained intensely with methyl

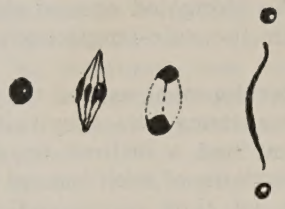

Fig. 1. Mitosis in *S. dreissenae* Dobrz.

green; they seemed to correspond to the chromosomes or to their agglomerations. This was the stage of prophase. The chromatine elements agglomerated to form a blade in the middle of the Mi while achromatinous agglomerations occurred on both sides of the blade; these agglomerations were stained badly with I. H., not at all with methyl green, and intensely with pyronin. Those portions corresponded to the karyokinetic spindle. This was the stage of metaphase. The present writer found on the contrary no division stages corresponding to anaphase which probably lasted for a relatively short period of time which was difficult to fix. However, the stage of late telophase often occurred in the material. During the stage of telophase, reconstruction of the filial Mi interphasal structure was observed. Generally, the process of mitosis corresponded exactly to the description of that in *Bursaria truncatella* O. F. Müll. given by Poljansky 1933. Similarly as in *Bursaria*, the bridge connecting the filial Mi and marking the track of their dispersing was maintained for a long period of time. One of the Mi remained in the apical part to make part of the nuclear apparatus of the bud which was to be formed later while the other slipped down (along the Ma) to the cytoplasmatic territory at the base (Fig. 1).

The problem of Ma duplication is not clear. Normally Ma divided by contraction to form two equal parts spherical in shape. Usually, the division plane run obliquely relative to the sole while the upper Ma formed a part of the bud which was being formed. The present authoress observed specimens with a scarcely marked division furrow in which, nevertheless, two fully formed Ma were present. Ma division was, on the contrary, delayed in other cases with the division furrow cutting off a part of the Ma of the maternal specimen. Here, the cortical processes preceded the division of the Ma and even more than this: they seemed either to provoke it or to speed it up mechanically. The Ma of the bud thus formed was stick-like in shape becoming thinner towards the suçoir while the Ma of the maternal specimen was irregular in shape. In cases like this, the two Ma remained connected to each other by means of a strand passing through the sucker of the bud during a long period of time.

The bud that was tearing itself off exhibited merely a small scar corresponding to the zone of the suçoir; the scar was marked solely by the lack of a few meshes in the network of the silver absorbing system. The rest of the body was uniformely covered with the network. It attached itself to the gill epithelium or to the mantle of the cavity wall within the pointed

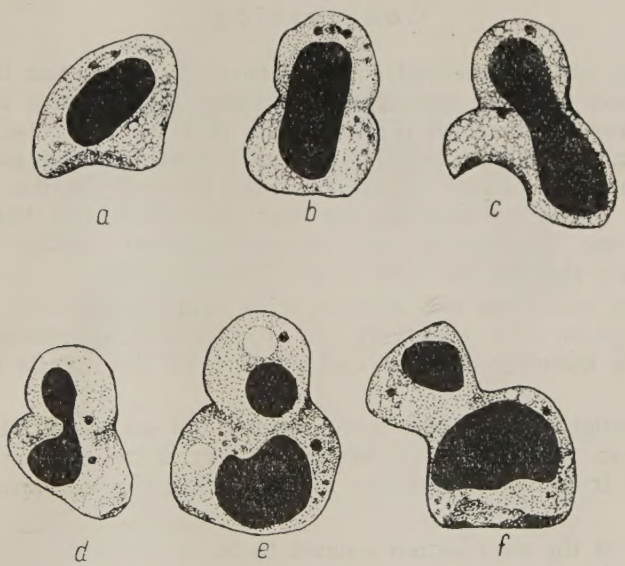


Fig. 2. Budding in *S. dreissenae*. a — mitosis of Mi; b — division of Mi; cortical scission before dividing of Ma; c — next stage of former; daughtery Mi are separated; d — cortical scission induces division of Ma; e — division of Ma preceeding cortical scission; C. V. are double; f — abnormal, lateral budding (drawing, after photomicrographs — I. H.)

sucker, and sole formation set in beginning at the point of attachment and extending along the body surface which was free from basal granules. All four rows of the left system (after Chatton et Lwoff 1950) touched directly the sole which was being formed and thus developed asymmetrically. In the course of further growth, this asymmetry became more marked as the ciliate became twisted. This resulted in a sharp curving of both of the systems in clockwise direction.

If the conventional orientation of *Ancistrocomidae* given by Chatton et Lwoff 1949 be applied to the bud, then the part of ectoplasm transforming into the sole will correspond to the antero-dorsal side. The process of the sole formation was accompanied by atrophy of the network of the silver absorbing system and by a marked granulation of those portions. The zone devoid of the net of the silver absorbing system was usually somewhat larger that that of the sole proper which had its distinct outline. Thus, the sole was usually bordered with transparent ectoplasm with the silver absor-

bing network, gradually disappearing towards the sole. The present authoress was not able to find the answer to the question as to the destiny of those fragments of the old sole as might be enclosed into the bud as a result of abnormal lateral budding (Fig. 2f, Phot. 10).

## Conjugation

The present writer observed conjugation of *S. dreissenae* in abundantly infected molluscs on three occasions: in October, in November and in March. Conjugation was universal in character, i.e. that the majority of specimens parasitizing on one mollusc conjugated simultaneously. Conjugation — or at least its preliminary phases — was observad not only in the fully mature *S. dreissenae* (their maturity degree was determined on the grounds of their dimensions, proportions and the arrangement of the rows of the silver absorbing system) but also in the juvenile ones.

The present authoress was able to trace some cojugations phases using the contrast phase and observing the conjugating specimens in smears in considerable quantity of slime and liquid from the mantle cavity of the mollusc.

The remaining observations were carried out on silver stained preparations, and on those fixed in Schaudinn liquid and stained with I. H. Unfortunately it proved impossible to trace back the chromosome changes in Mi.

The stages of the conjugation seemed to be as follows:

1. The agglutination stage or the protozoans tendency to stick together. Often large groups of specimens sticking together in sterile agglomerations occurred in silver stained smears or in hematoxylin ones. This phenomenon seemed not to be accidental as the same could be observed in vivo. Besides, these observations were in harmony with conjugation phases of many tribes in culture, e.g., studies of *Paramecium bursaria* (Ehrbg.) and *Tetrahymena pyriformis* (Ehrbg.) conjugation — Lwoff 1947. Taking into account the absence of any active movement in *S. dreissenae*, this seemed to proceed as follows: the protozoans sole detached from the base and the water current floated them slowly into the slime. Permanent agglutination occurred whenever they came into contact with other specimens.

2. The stage when the proper position of the specimens towards each other is assumed. The agglutinating specimens were attached to each other only by means of the processes corresponding to the suçoir in most cases (see morphological considerations on *Sphenophryidae* — Chatton et Lwoff 1931, 1950).

Both, the suçoir and the process of the sole exhibited a considerable degree of elasticity in *Sphenophrya dreissenae* so that the shape of both the suçoir and the process could be elongated or hooked which facilitated the clinging to each other of the two specimens. It proved impossible to trace back the course of the further stage in vivo. The flow of water or perhaps the sticky character of the pellicle and especially that of the sole surface seemed

to cause twisting of the specimens in such a way that they were sticking to each other with their soles.

Thus, as a result of the body twisting, the flank side (in which the two systems of basal corpuscle rows are visible) of one specimens joints together with that side of the other one which is devoid of basal corpuscules (properly speaking that surface is not completely devoid of corpuscules as it contains

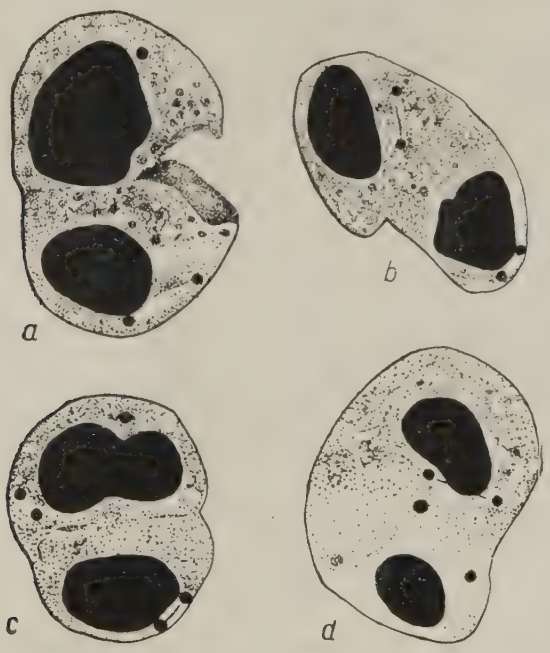


Fig. 3. Conjugation in *S. dreissenae*. a — first division of Mi; many food reserves in cytoplasm; b — the soles of both partners are completely fused; Mi in both partners in the first maturation division; c — next stage; Mi of one partner probably in the second maturation division; d — conjugation; second maturation division in one specimen (there are three Mi, two degenerating); second partner has two Mi (probably pronuclei); Mi are gathered in the zone of exchange of pronuclei. Cytoplasm clear and transparent (drawings after photomicrographs — I. H.)

fragments of rows of the right five-row-system of basal corpuscles which appeared at this side as a result of the body torsion). The protozoans remained for a long period in this stage: about 1 to 1.5 hours under the not natural conditions of the in vivo observation. The relatively long duration of conjugation (i.e. of that stage when the ciliates are not attached to epithelium with their surface and, consequently, may be very easily washed away) ought to be emphasized in this connection. This can possibly occur when the mollusc has shut during the autumn and winter months with their unsignificant water flow and the slime probably increasing in quantity. The conjugating specimens arrangement opposite to each other was quite different than

in *Sphenophrya dosiniae* (Chatton et Lwoff 1921, 1931, 1950, Miassnikowa 1930, Raabe 1938) in which all of the authors were unanimous to observe the conjugating individuals being in a linear arrangement. Also the studies on *Sphenophrya sphaerii* carried out by Miassnikowa 1930 and Raabe 1949 showed the conjugating individuals to be arranged linearly. There are no data available on the conjugating specimen arrangement

Fig. 4. Sketch of the reciprocal disposition of conjugating individuals of *Sphenophrya dosiniae* (after Raabe 1938), of *S. dreissenae* (orig.), and of *Pelecyophrya tapetis* (after Chatton et Lwoff 1950)

of *Gargarius gargarius* and *Lwoffia cilifera*. On the contrary, the observations concerning conjugation of *Pelecyophrya tapetis* Chatton et Lwoff 1922 are very interesting. The above mentioned authors confirmed that conjugation occurred between individuals approximately equal in size „qui s'accolent par leur pôle postérieur et se soudent ensuite par un secteur longitudinal plus ou moins étendu", Chatton et Lwoff 1950. This might be not a linear but a symmetrical arrangement of individuals. However, an accurate and efficient comparison to *Sphenophrya dreissenae* has been impossible due to lack of further data. The fact that conjugating *Sph. dreissenae* stick to each other with their soles may possibly prove the latter to be precisely the ventral portions of the bud, i.e. those parts of the pellicle where formerly the mouth of the ancestors of *Ancistrocomidea* was situated. The demarcation line between the two individuals was gradually disappearing during a period of about one hour. This phenomenon appeared first at the suçoir region subsequently spreading out gradually into the two soles. Sometimes, however, irrespective of the suçoir and the processes being already confounded at the opposite side of the body there remained a part of slime between the two individuals. Such a „vacuole" is gradually squeezed out till full integration of the two protozoans is achieved. The conjugating individuals might differ in size, however specimens equal in length conjugated on most occasions, similarly as it was the case with other *Sphenophrya* (Chatton et Lwoff 1950). In case of different sizes of the conjugating individuals, the soles adjusted themselves to each other owing to the body plasticity of the two partners. During the last stage of the two partners' union, a common network of silver absorbing system was being formed at the limit between them. This phenomenon has been described by Raabe 1949 in *Sphenophrya sphaerii*. There was no difference between the meshes of that network and the structure of the remaining body. However, rarefied basal corpuscles and dispersed parts of kineties were usually observed in the region adjacent to the sole. These observations are in perfect accordance with

the findings of Raabe 1938, 1947, 1949. The conjugating couples resembled a sphere at this stage.

3. The conjugation process. The present authoress traced these processes on preserved and I. H. stained material only. No changes in the Ma were observed during the whole period of conjugation. The Ma was compact and changes in its shape could be easily connected with its displacement during conjugation. The presence of the unchanged Ma was confirmed in other protozoans too, for example in *Cryptochilum echini* (Maupas) described by Dain 1930, or in *Ancistrum mytili* (De Morgan) described by Kidder 1930. However, fragmentation and elongation of the Ma as early as at the preliminary conjugation phase was confirmed in *Pelecyophrya tapetis* (Chatton et Lwoff 1922) as well as in *Sphenophrya dosiniae* Ch., Lw. (Chatton et Lwoff 1921, 1950 and Raabe 1938).

The Mi was situated in the apical part of the individuals during the preliminary conjugation phase. The first division of the Mi occurred when the soles of the conjugating individuals did not yet completely amalgamate; this seemed to last for a relatively long period for stages of division of the Mi still occurred in specimens with fully amalgamated soles.

Two filial Mi displace themselves, namely: they go round the Ma, along its both sides, sliding on the homogenous cytoplasm zone around it thus approaching the place where formerly the sole was situated. On some occasions the two Mi float one after the other along the same Ma side. Their migration is easily detected owing to the traces of the karyokinetic spindle marking the way they pass. At that stage the Ma of the partners are shifting into the apical parts of cells. The cytoplasmatic zone is differentiating distinctly and further nuclear changes are going on in it. Occurrence of such a zone was confirmed in other protozoa too, e.g. in *Bursaria truncatella* by Poljansky 1934.

Soon, the second division occurs in the two partners and there are 4 Mi in each cell. The present authoress observed specimens at this stage on several occasions. However, full synchronization of division processes of the Mi occurred seldom in both conjugating individuals. Some of these Mi had a loosened chromatin and exhibited marks of degeneration. The present authoress cannot confirm the occurrence of the third division with full certainty due to the chromosome numbers not being established; still, this should be expected. On one occasion, however, the authoress found conjugating individuals one of which had three Mi; two of them were degenerating while the partner had two distinct Mi which probably already became the pronuclei.

The present authoress was able to confirm beyond all question the passing of the transitory pronucleus into the partner; this was possible thanks to the characteristic darkening of I. H. stained cytoplasm which marked the way of migration. Further stages are impossible to trace without any "in vivo" observations.

In describing the budding process, examples of anomaly were given and an attempt was made at explaining them. This thesis was corroborated by the fact of two conjugating individuals one of which had five rows of right system basal corpuscles while the other had only four of them.

The present authoress confirmed cytoplasm changes during conjugation in *Sphenophrya dreissenae* which was in accordance with Poljansky's 1933 study on *Bursaria truncatella* O. F. Müller. Plasm of the conjugating individuals was considerably vacuolized and granular during the first phases while it became transparent and homogenous during conjugation. „Bei den Konjuganten verliert das Entoplasma seine Vakuolen und wird fast homogen" (Poljansky 1933). This may be observed even in the photographs. The phenomenon of changes in cytoplasm is usually related with the not functioning mouth apparatus, i.e., with gradual spending of accumulated reserve materials, e.g. of glicogen. The occurrence of such a phenomenon in *Sphenophrya dreissenae* proves that the latter is not feeding during conjugation; especially so, if we consider the fact of the two individuals sticking to each other with their sole, i.e., with that part which is likely to play the main part in feeding.

## The structure and formation of the sole

The formation of the sole in *Sphenophryidae* as a differentiated adhesive surface is related to the transformation of the tomits suçoir together with the adjacent pellicle portions.

The suçoir, or the part of the bud corresponding to it, is formed at the limits between the primite and opistor during the division or budding period. Consequently, there is nothing unusual in the *Sphenophryidae* sole being generally formed de novo, and not as a result of division of the sole of the mother specimen.

In seeking the answer to the problem as to which pellicle part is responsible for the formation of the adhesive surface, the adult form is to be properly oriented supposing the plane of division (which generally is transversal in respect to the kineties) as horizontal.

Below, the process of the sole formation in all *Sphenophryidae* species is described according to the progressive gradual disappearing of cilia. The latter phenomenon seems to be a distinct feature of parasitic adaptability, i.e. a substantial developmental tendency justifying classification. On the other hand, however, the reservation ought to be made that the series given below is not considered by the present authoress as some real evolutional series; quite on the contrary, within the family of *Sphenophryidae*, the process of evolution was certainly not a one-feature-evolution, but a many--feature one, radiation-like in character.

Here now is the series:

1. *Lwoffia cilifera* Kozloff 1955 possesses cilia at the trophical stage as well as at the juvenile one. This is a species in which the typical division can be observed and the specimens thus formed do not differ from each other. *Lwoffia cilifera* is a protozoan with two systems of basal corpuscle rows. Admitting body orientation after Kozloff, the right hand system consists of 5 parallel kineties while the left one numbers 2 + 3 or 2 + 2. Generally speaking, the *Lwoffia cilifera* structure resembles a representative of the family *Ancistrocomidae* flattened along the plane running between the two systems of kineties. Two halfcircular edges are thus formed, the one

being parallel and the other perpendicular in respect to the kineties. The beginnings of the sole formation may be observed as early as at the division period. This process causes some disturbances in the division furrow and brings about the bending of the kineties. The sole covers a region corresponding to the suçoir, together with the lateral edge which is parallel to the line of kineties and separates the two systems. The degree of development of the attachment surface has been described by Kozloff 1955 thus: "The attachment surface of *Lwoffia* is more extensive than that of *Pelecyophrya*, and is disposed on those portions of the body which correspond to the anterior end and anterior part of the dorsal surface in *Pelecyophrya*. The suçoir of *Lwoffia* resembles that of *Pelecyophrya* in having a broad funnel-shaped outline when viewed from the right or left side of body. The margins of the suçoir may be discerned in some specimens stained with I. H., and individuals impregnated with protargol show them quite regularly. The suçoir becomes broader antero-dorsally and its anterior margin may often be traced as far as the attachment surface".

2. *Pelecyophrya tapetis* Ch. Lwoff 1922. The trophic form is covered with ciliae sporadically only, while the bud is ciliated. Chatton et Lwof 1922 pointed out that this was a species in which the division already had the characters of budding. A ciliated tomit is formed as a result of intensive development of pellicle in the region of the division furrow which deepens, thus cutting out the conical front part of the bud. The two-system bud (5 and 2 + 2) is also subject to flattening alongside the plane stretching between the two systems of kineties. A strong suçoir is situated on the area of the attachment surface which is formed on the lateral erge. The tomit — tomont size ratio equals nearly one.

The sole dimensions and character are indicated on the grounds of observations of fixed preparations from the Monaco material only. The sole is elastic and elliptic in shape. Its edge will intensely stain with iron hematoxylin. There often occurs a cytoplasm furbelow surrounding the sole.

3. *Gargarius gargarius* Ch. Lwoff 1934. Many features of this form differ essentially from the remaining species. Cilia are lacking in the maternal organism while they are always present in the bud which is slightly flattened, resembling in shape typical *Ancistrocomidae*. Both systems of kineties (4 — 6 and 2 + 2) are situated at the same side of body. *Gargarius gargarius* does not form any attachment surface; however, an excessively strong development of the suçoir is observed. The latter resembles an elongated furrow bent asymmetrically, i.e. towards the left edge according to Chatton et Lwoff. Oblique division occurs owing to the lacking attachment surface, a strongly emphasized suçoir being only present; such division is characteristic of *Ancistrocomidae;* however, it is somewhat modified by the early appearing suçoir structure and anlagen of crest. The filial specimens are approximately equal in size after division.

4. *Sphenophrya dosiniae* Chatton et Lwoff 1921. The trophic specimen is devoid of cilia with the two systems of kineties (4 — 6 and 2 — 3) at the same side of the flattened body. Typical budding occurs. The mechanism of the development of the ciliated bud was examined by Chatton et Lwoff 1921, 1931, Miassnikowa 1930 and Raabe 1938, 1949. The latter author confronted and compared the *Sphenophrya dosiniae* deve-

lopment to that of *Sphenophrya spherii* and indicated the particular portions of the bud as will subsequently form the sole. The body of *Sphenophrya dosiniae* is distincly flattened. The sole is keel-like in character which permits penetrating among the epithelium cells of the host. The sole thus forms the two flank surfaces of the keel. There is a distinct difference between the silver absorbing system of the whole body and that of the sole which stains with I. H. more intensely; the silver absorbing network of the sole has meshes with higher granulation degree. In most cases, however, the network occurs but for a few exceptions with fully differentiated homogenous sole surface similarly as in *Sphenophrya sphaerii* or *Sphenophrya dreissenae.*

4a. In connection with the description of a separated species *Sphenophrya cardii* Chatton et Lwoff 1950, the present authoress carried out several measurements and observations on ciliate taken from *Cardium edule* and, for comparison, on *Sphenophrya dosiniae* from *Mya arenaria* from the material of Prof. Z. Raabe who collected it in the Gdańsk Bay of the Baltic Sea. Those measurements and observations enable us to confirm an absolute accordance in such features as the mechanism of development, the character of sole as well as a considerable degree of coincidence of the number of kineties. The differentiation of a new species seems not to be justified, at least not at the grounds of characteristics known so far. However, considering the biometric data pointing to differences in body shape and to some distinctive features in the population from *Cardium edule* in respect to the kineties number, it would seem correct to adapt the point of view of Raabe 1938 who differentiated and defined *Cardium edule* populations as mere *Sphenophrya dosiniae* f. *cardii* (See Table I).

5. *Sphenophrya minor* Poljansky 1951 from *Cardium* sp. from Vladivostok, USSR. A species with a very high number of kineties: 7 — 9 and 2 + 2. However, the same system of kineties sporadically occurs in *Sphenophrya sphaerii,* and the quantity of 7 right-side kineties occurs in *S. dosiniae* too. The species definition is not sufficient and the confrontation of features distinguishing it from *S. dosiniae* and *S. sphaerii* is casual. Data on the sole formation are lacking.

6. *Sphenophrya sphaerii* Miassnikowa 1930. Trophic specimen with no cilia, with both of the two systems (5 — 6 and 2 + 2) on the same side of body. The typical conical cutting off of bud similarly as in *S. dosiniae.* Raabe 1949 has described the mechanism of the distinctly differentiated and fully integrated sole. Out of all *Sphenophryidae* this is the species with the most differentiated sole. The latter is markedly wider than in *S. dosiniae* and bears the character of an attachment surface.

7. *Sphenophrya dreissenae* Dobrzańska 1958. In this species a typical budding occurs which is related to a preparatory process (partial despiralization). The sole is formed as a result of transformation of the suçoir together with the adjacent portions of the pellicle devoid of the kineties systems. Yet, the sole is somewhat shifted towards the 2 + 2 system whereas it is distant from the 5 spiral kineties of the second system. The sole forms already on the ground of the silver absorbing system of the bud which is becoming diluted. That is why a zone of diluted silver absorbing system often occurs between the significantly differentiated sole and the remaining pellicle.

*Sph. dreissenae* is a very specialized species with total reduction of the tomont cilia, whereas cilia occur only sporadically on the bud.

## General remarks on phylogenesis and relationship within *Sphenophryidae*

1. General conformity in tomit structure may be established when comparing different genera of *Sphenophryidae* to each other. The tomit represents that form in the developmental cycle which is characterized by ancestral features; this ensues from the findings of Chatton et Lwoff 1935—1936 who studied *Apostomea* and *Conidophryidae*, as well as from those of Guilcher 1951 who studies the processes of budding in *Suctoria* and *Chonotricha*. The tomit in all *Sphenophryidae* genera proved to have the same arrangement of kineties which more or less corresponded to the pattern 2 + 2 and 5.

2. Constancy of the kineties arrangement represents a particularly important argument in phylogenetic considerations; however, it should be kept in mind that the number of rows is not an adaptation feature in *Sphenophryidae*.

Another constant feature which seems to be characteristic of all *Sphenophryidae* (*Sph. minor*?) is the occurrence of "corps énigmatiques" or some other elements strenghtening the suçoir. Those elements resemble the skeleton of the suçoir in some *Ancistrocomidae*, for instance in *Hypocomidium fabius* Raabe 1938.

3. Examining the development of *Sphenophrya sphaerii* and *S. dossiniae*, Raabe 1949 pointed out that the tomit is identical or almost identical in structure with the present day representative of *Hypocomatidum sphaerii* Jarocki et Raabe 1933 (*Ancistrocomidae*) which occurs commonly both in *Pisidium* sp. sp. and in *Spherium* sp. sp. It is peculiar that no simultaneous invasion of molluscs by both *Sphenophrya sphaerii* and *Hypocomatidium sphaerii* was ever noted to date. Yet, disregarding the two genera resembling each other, it ought to be possible to diagnose simultaneous invasion because of the difference in dimensions of *S. sphaerii* tomite and *H. sphaerii*.

Another species of the genus *Hypocomatidium*, *H. jarockii* Chatton et Lwoff 1950, is a sea form which is more primitive than *H. sphaerii*. The principal pattern of cilia lays within the limits of variability of the kineties arrangements in *Sphenophryidae* and is 3 + 2 and 6. Consequently, the structure of one of these species seems to correspond to that of the hypothetic form of the ancestor of *Sphenophryidae*.

4. In all of *Sphenophryidae*, the division plane is arch-like bent during division similarly as it is in *Ancistrocomidae*. In species propagating themselves by budding, the division furrow is bent more markedly. This, in the representatives of the genus *Sphenophrya*, results in a circle forming during the budding which cuts out a part of the cytoplasm. The cutting out of the bud is related to the dynamic net development (Raabe 1949) cutting out the front part of the bud which is sunk conically into the body of the mother specimen.

5. The process of formation of the attachment surface is related to the disappearance of cilia; the attachment surface has differentiated most markedly

Tab

Comparison of the features

| Name of species | Lwoffia cilifera Kozloff 1955 | Pelecyophrya tapetis Ch.Lw.1922 | Gargarius gargarius Ch.Lw. 1934 | Sphenophrya sphaerii Miassnikowa 1930 |
|---|---|---|---|---|
| Place | Caloosahatchee River Fort Myers-U.S.A. | Roscoff Baniuls sur Mer-France | Sete - France | Neva River Golf of Finland-U.S.S.R. |
| Typical host | Brachidontes recurvus Raf. | Tapes aureus (Gmel.) | Mytilus edulis L. | Sphaerium corneum L. |
| Other place host and Synonimes | | Monaco (Raabe 1936) Tapes sp.(Inedit.) Pelecyophrya tapetis | Hel-Poland (Raabe 1935) Mytilus edulis L. Rhynchophrya cristallina | Lake Żarnowieckie-Poland (Raabe 1949) Strasbourg-France (Ch.Lw.1950) Warszawa-Poland (Dobrzańska 1961) Sphaerium corneum L. |
| Environment | Lagoon marshy | Sea with high-salt contains | Sea, and sea with fresh-water inlet | Rivers, lakes, chanals fresh-waters |
| Cili- embryo etion adult | Ciliated Ciliated | Ciliated Nonciliated (as arule) | Ciliated Nonciliated | Ciliated Nonciliated |
| No.of kineties left- right syst. | 3 + 2 5 (3 + 3) | 2 + 2 5 | 2 + 2 4 (4 - 6) | 2 + 2 5 (2+1)-(3+3) (4-8) |
| Dimension basis × height | 14 × 16 - 25 × 29 (Kozloff 1955) | 12 × 16 - 15 × 30 (Dobrzańska 1961) | 15 × 40 - 15 × 50 (Raabe 1935) | 10 × 25 - 35 × 35 Miassnikowa 1930 13 × 50 - 15 × 80 Raabe 1949 |
| Adhesion degree basis:height | 0.8 - 0.9 | 0.6 - 0.8 | 2.8 - 3.3 | 2.5 - 5.1 |
| Adhesion surface | Sucker and funnel | Suçoir, siphon | Suçoir, siphon | Sole |
| "Corps enigmatiques" | Primordium of sucker (Kozloff 1955) | Corps siderophiles (Ch.Lw.1922) | Costules du suçoir (Ch.Lw.1934) | Rätzelhafte Gebilde (Miassnikowa 1930) |
| C.V. | Present | Absent | Absent | Present |
| Mode of nutrition | Osmose | Phagocytose | Phagocytose (Ch. Lw.) Osmose (Raabe) | Phagocytose |
| Length of cilia | 8 - 9 | - | - | - |
| Dimension of Ma and Mi | | 6 - 10 × 6 - 8 2 - 3 | 25 × 6 5 | 50 × 6 |
| Multiplication | Division | Division | Division-Budding | Budding |
| Dimension of bud | - | - | 28 × 16 | 17 × 14 |

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of Sphenophryid species

| Sphenophrya dosiniae Ch.Lw.1921 | Sphenophrya dosiniae f.cardii Raabe 1938 | Sphenophrya minor Poljański 1951 | Sphenophrya dreissenae Dobrzańska 1958 |
|---|---|---|---|
| Roscoff-France<br>Woods Hole-U.S.A. | Hel-Poland | Vladivostok-U.S.S.R. | Poland |
| Venus ovata Pennant<br>Corbula gibba Oliv.<br>Dosinia exoleta L.<br>Mactra solidissima L.<br>Mya arenaria L. | Cardium edule L. | Cardium sp. | Dreissena polymorpha Pall. |
| Kandalakschka-U.S.S.R.<br>(Miassnikowa 1931)<br>Mya truncata L.<br>Sphenophrya myae<br>Hel-Poland<br>(Raabe 1934)<br>Mya arenaria L. | Roscoff-France<br>(Ch.Lw.1950)<br>Cardium edule L.<br>Sphenophrya cardii | | |
| Sea and sea with freshwater inlet | Sea, and sea with freshwater inlet | - | Lakes, rivers fresh-waters |
| Ciliated<br>Nonciliated | Ciliated<br>Nonciliated | - | Noncilated (as arule)<br>Nonciliated |
| 1 + 2 1 + 4<br>(2 + 2) (1 + 5) | 2 + 2 1 + 5<br>(2+1)-(2+3) (1+4)-(1+6) | 2 + 2 8<br>(1 + 2) (7 - 9) | 2 + 2 5<br>(3 - 5) |
| 15 × 120 - 20 × 120<br>Ch.Lw. 1921<br>6 × 125 - 11 × 125<br>Miassnikowa 1951<br>7 × 120 - 12 × 120<br>Raabe 1938 | 7 × 80 - 12 × 80<br>Raabe 1938<br>5 × 120 - 7 × 120<br>Raabe 1949 | 13 × 30 - 20 × 85<br>Poljanskij 1951 | 24 × 22 - 34 × 40<br>Dobrzańska 1958 |
| 6.0 - 21.0 | 6.6 - 24.0 | 2.6 - 4.2 | 0.9 - 1.2 |
| Sole | Sole | Sole | Sole |
| Baguettes tubulaires<br>(Ch.Lw.1921) | Baguettes tubulaires<br>(Ch.Lw.1950) | - | Hyalinous elipses<br>Dobrzańska 1961 |
| Present | Present | - | Present |
| Phagocytose<br>(Ch.Lw. 1950)<br>Osmose<br>(Polianskij 1951) | Phagocytose | Phagocytose | Osmose (pinocytose?) |
| 6 | - | - | 5 |
| 40 × 2 - 4<br>2 - 3 | 38 × 2 - 4<br>2 - 3 | 10 | 9 - 12 × 5 - 10<br>3 - 4 |
| Budding | Budding | Budding | Budding |
| 20 × 12 | 20 × 9 | - | 10 × 7 |

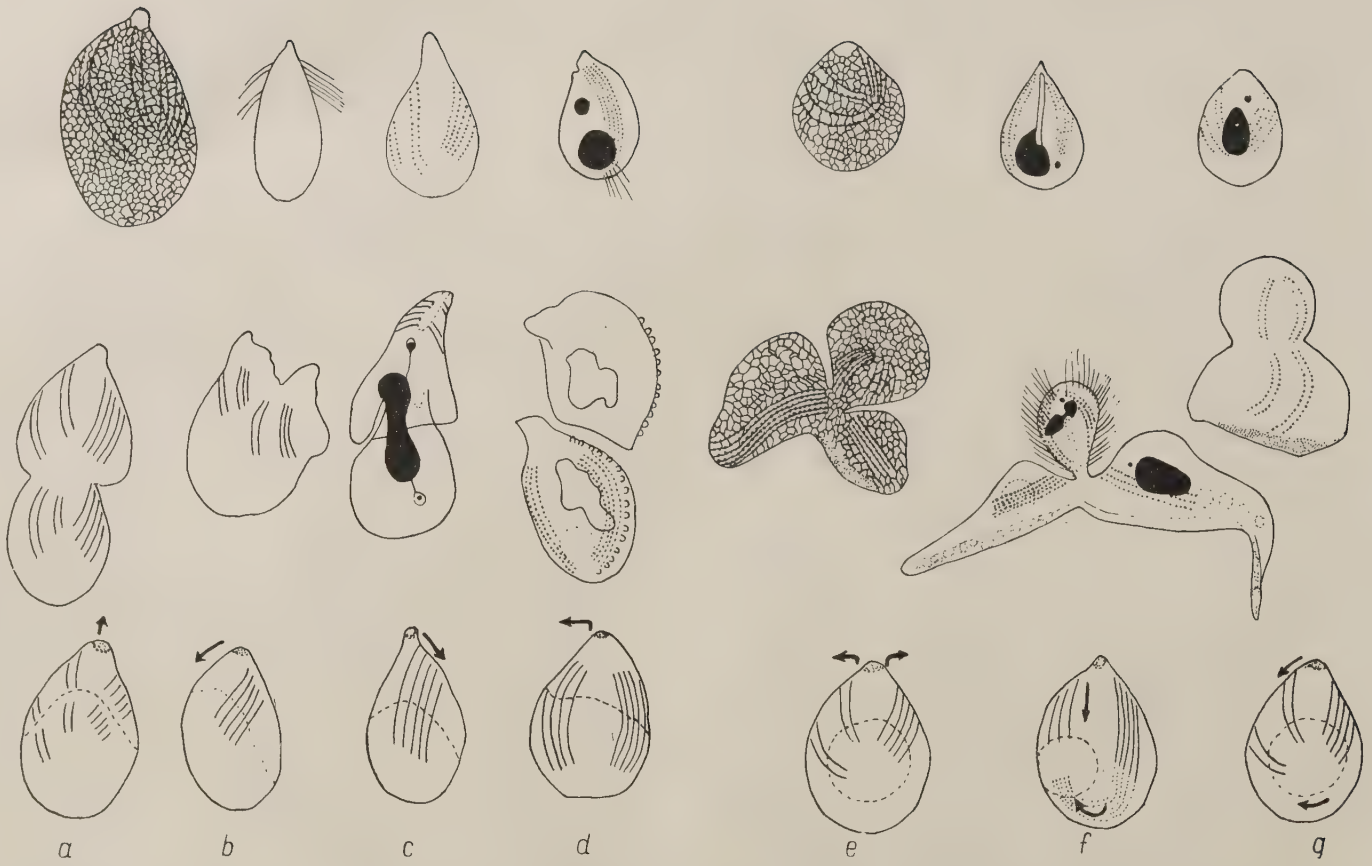


Fig. 5. Pattern of development of bud of *Ancistrocomidae*-like structure in different species within *Sphenophryidae* (comparison of bud, dividing form, and bud pattern). a — *Hypocomatidium sphaerii* after Raabe 1949, and Raabe not edit., modified; b — *Lwoffia cilifera* after Kozloff (cross section diagram of protozoan instead of the bud); c — *Pelecyophrya tapetis* after Chatton et Lwoff 1950, modified; d — *Gargarius gargarius* after Chatton et Lwoff 1950; e — *Sphenophrya sphaerii* after Raabe 1949; f — *S. dosiniae* after Raabe 1938; g — *S. dreissenae* after a photograph of author. Symbols: arrow — direction of development of attachment surface; interrupted line — outline of the furrow of future division marked on the sketch of bud; points — sucoir of bud

in species of genus *Sphenophrya*. The wide and multiform developmental facilities of the suçoir as an attachment organ result in various developmental mechanisms which sometimes are even similar in effect: e.g. the similar trophont structure in *Sphenophrya sphaerii* and *S. dosiniae* (Raabe 1949).

There occurs a tendency towards body spiralization in *Sph. dreissenae*.

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Taking into consideration all the above observations, the *Sphenophryidae* may be confirmed to constitute a natural group of closely congeneric protozoans which doubtlessly derive from tribes of *Ancistrocomidae*, already very advanced in their adaptation to parasitizing. There occurred some specialization within *Ancistrocomidae* resulting in formation of a large number of species similar in the pattern of structure and merely differing in the arrangement of cilia and in the extent of its reduction. Highly specialized forms of *Ancistrocomidae* are represented by the present day species of the genera *Hypocomatidium*, *Hypocomidium* and *Enerthrecoma*. There is little probability in a possible tracing back of *Sphenophryidae* back to forms resembling *Hypocomidium* and *Enerthrecoma* alongside with *Hypocomatidium* (as suggested by Chatton et Lwoff 1950) because of the *Sphenophryidae* group being very compact. The resemblance of sucoir of *Pelecyophrya tapetis* and of *Hypocomidium fabius* may be convergent in character. Thus, alongside with one specialization series where the genera of *Ancistrocomidae* family (*Goniocoma*, *Anisocomides*, and *Hypocomatidium*) illustrate the various stages of one developmental tendency, the forms resembling *Hypocomatidium* have produced a group of ciliates exhibiting a new developmental tendency emphasized by various intensity, namely: the forming of attachment surface related to the losing of cilia.

The already markedly specialized forms serving as point of departure for a whole group of animals thanks to the formation of a new adaptation mechanism would therefore present an interesting example. Further evolution made it possible for the new tendency to turn true in various ways, and the evolution of the group became radiant in character.

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## LITERATURE


1. Chatton E., Lwoff A. — Sur une famille nouvelle des Acinétiens, les Sphénophryjdés, adaptés aux branchies des Mollousques acéphales. C. R. Acad. sci., 173, 1921.
2. Chatton E., Lwoff A. — Sur l'évolution des Infusoires des Lamellibranches. Le genre *Pelecyophrya*, intermédiaire entre Hypocomidés et Sphénophryidés. Bourgeonnement et conjugaison. C. R. Acad. sci., 175, 1922.
3. Chatton E., Lwoff A. — Sur l'évolution des Infusoires des Lamellibranches. Relations des Sphénophryidés avec les Hypocomidés. C. R. Acad. sci., 175, 1922.
4. Chatton E., Lwoff A., Wwoff M. — L'infraciliature de l'Infusoire thigmotriche *Sphenophrya dosiniae* Ch. et Lw. Topographie, structure et développement. C. R. Soc. biol., 107, 1931.
5. Chatton E., Brachon S. — Les relations du chondriome avec l'infraciliature chez divers ciliés. C. R. Soc. biol., 118, 1935.
6. Chatton E., Lwoff A. — Les Ciliés Apostomes. Arch. Zool. exp. gén., 77, 1935.
7. Chatton E., Lwoff A. — Les *Pilisuctoridae*, Ciliés parasites des poils secréteurs des Crustacés Edriophtalmes. Orientation et polarité chez les Infusoires. Bull. biol. France-Belg., 71, 1936.
8. Chatton E., Lwoff A. — Sur un cilié Thigmotriche nouveau: *Gargarius gargarius* n.g., n. sp. de *Mytilus edulis*. Bull. Soc. zool. France, 59, 1934.
9. Chatton E., Lwoff A. — Sur le suçoir des Infusoires Thigmotriches Rhynchoidés (*Hypocomidae* et *Sphenophryidae*) et sa genèse. C. R. Acad. sci., 209, 1939.
10. Chatton E., Iwoff A. — Recherches sur les ciliés Thigmotriches. Arch. Zool. exp. gén., 86, 1950.
11. Chatton E., Lwoff A. — Recherches sur les ciliés Thigmotriches. Arch. Zool. exp. gén., 86, 1950.
12. Dain L. — Die Konjugation von *Cryptochilum echini* Maupas. Arch. Protistenk., 70, 1930.
13. Dogiel V. — Obščaja protistologija. Moskva, 1951.
14. Dobrzańska J. — *Sphenophrya dreissenae* sp. n. (*Ciliata, Holotricha, Thigmotrichida*) living on the gill epithelium of *Dreissena polymorpha* Pall., 1754. Bull. Acad. Polon. Sci., 6, 1958.
15. Fauré-Fremiet E. — Étude sur les mitochondries des Protozoaires et des cellules sexuelles. Arch. Anat. Micr., 10, 1910.
16. Guilcher Y. — Contribution à l'étude des Ciliés Gemmipares, Chonotriches, et Tentaculiféres. Ann. Sci. Nat., 13, 1951.
17. Kidder G. — Studies on *Conchophthirus mytili* de Morgan. Arch. Protistenk., 79, 1933.
18. Kidder G. — On the genus *Ancistruma* Strand (*Ancistrum* Maupas). II. Biol. Bull., 64, 1933.
19. Kozloff N. — *Lwoffia cilifera* g. n. sp. n., a ciliated member of the family *Sphenophryidae*. Biol. Bull., 108, 1955.
20. Miassnikowa M. — *Sphenophrya sphaerii* ain neues Infusorium aus *Sphaerium corneum* L. Arch. Protistenk., 71, 1930.
21. Miassnikowa M. — Über einen Vertreter der Familie *Sphenophryidae* aus *May truncata* L. Arch. Pritistenk., 72, 1930.
22. Poljansky G. — Geschlechtprozesse bei *Bursaria truncatella* O. F. Müll. Arch. Protistenk., 81, 1933.
23. Poljanskij J. I. — O nekotorych parazitičeskich infuzoriach iz morskich molliuskov i goloturii. Parazit. Sbornik Zool. Inst. AN SSSR, 13, 1951.
24. Raabe Z. — *Rhynchophrya cristallina* g. n. sp. n. nouvelle forme de la famille des *Sphenophryidae* Chatton et Lwoff. Bull. Inst. Océan. Monaco, 676, 1935.

25. Raabe Z. — Weitere Untersuchungen an parasitischen Ciliaten aus dem polnischen Teil der Ostsee. Ann. Mus. Zool. Pol., 13, 1938.
26. Raabe Z. — Studies on the *Hysterocinetidae* Diesing (*Ciliata, Holotricha*). Ann. Mus. Zool. Pol., 14, 1949.
27. Raabe Z. — Recherches sur les ciliés Thigmotriches (*Thigmotricha*) Ch. Lw. III. Ann. Univ. M. Curie-Sklod., C, 4, 1949.
28. Stevens N. M. — Studies on Ciliate *Infusoria*. Proc. Calif. Acad. Sci. (3), 3, Zool., 1901

## STRESZCZENIE

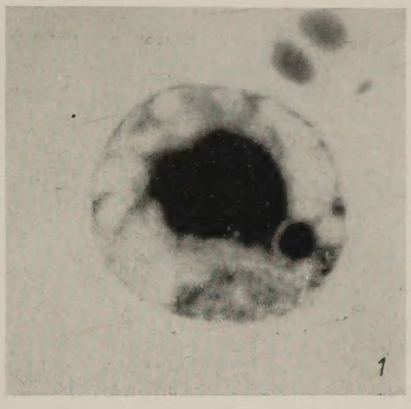
Autorka podaje nowe dane dotyczące występowania, morfologii i morfogenezy orzęska *Sphenophrya dreissenae* Dobrzańska 1958 (*Holotricha, Thigmotrichida*) występującego na nabłonku oddechowym *Dreissena polymorpha* Pall. *Sphenophrya dreissenae* powszechnie występuje w małżach zarówno pochodzących z jezior jak i rzek w wielu punktach Polski.

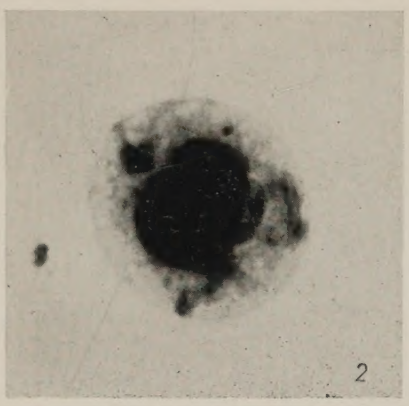
Podany jest opis przemian morfologicznych, związanych z pączkowaniem, oraz obserwacje dotyczące koniugacji. Dane te wraz z opisem poszczególnych struktur orzęska autorka zestawia z innymi gatunkami z rodziny *Sphenophryidae*, rozważając stosunki filogenetyczne wewnątrz tej grupy.

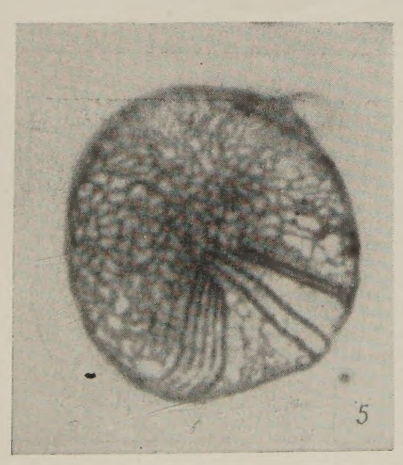
## EXPLANATION OF PLATES I—VI

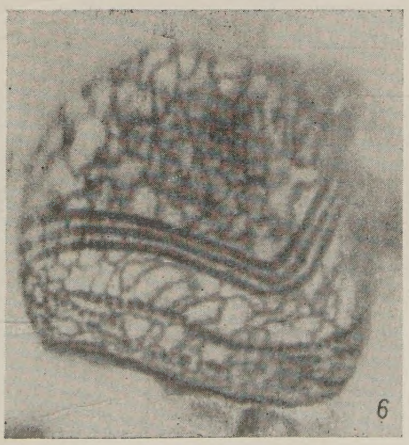
*Sphenophrya dreissenae* Dobrzańska; photomicrographs:

Phot. 1. Structure of cytoplasm, I. H.
Phot. 2. "Corps enigmatiques", I. H.
Phot. 3. Mitosis of Mi-telophase, I. H.
Phot. 4. "Granules erratiques" and character of "dynamic net", silver impregnation.
Phot. 5. Apex and spiralization of kineties, silver impregnation.
Phot. 6. Curving of kineties in predivision stage — "granules erratiques" (only three kineties of right system), silver impregnation.
Phot. 7. Predivision stage, silver impregnation.
Phot. 8. Dividing furrow formation, silver impregnation.
Phot. 9. Dividing furrow formation, silver impregnation.
Phot. 10. Lateral budding, silver impregnation.
Phot. 11. Sole formation of embryo, silver impregnation.
Phot. 12. Sole formation of embryo, silver impregnation.
Phot. 13. First stage of torsion of kineties of embryo, silver impregnation.
Phot. 14. Agglutination of pre-conjugants, silver impregnation.
Phot. 15. Attachment of individuals by „suçoir", silver impregnation.
Phot. 16. First stage of joining pre-conjugants by soles, silver impregnation.
Phot. 17. Disappearing of demarcation line between two individuals, silver impregnation.
Phot. 18. Squeezing of „vacuole" between two individuals, silver impregnation.
Phot. 19. First maturation division of Mi, phase contrast in vivo.
Phot. 20. Structure of sole of *Sph. dosiniae*, silver impregnation.
Phot. 21. Cutting out of a bud of *Sph. dosiniae*, silver impregnation.
Phot. 22. Structure of sole of *Sph. sphaerii*, silver impregnation.
Phot. 23. Sole formation of embryo of *Sph. sphaerii*, silver impregnation.



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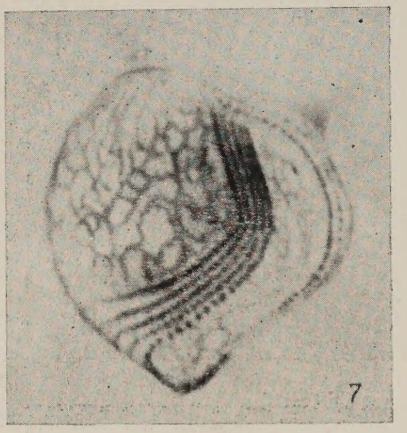


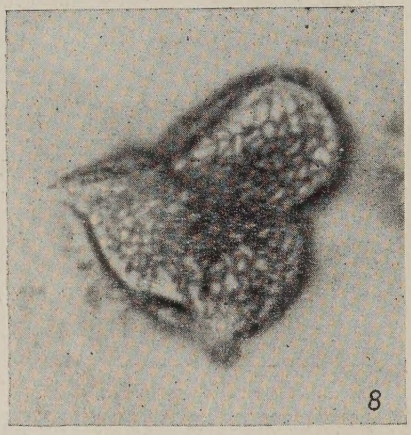


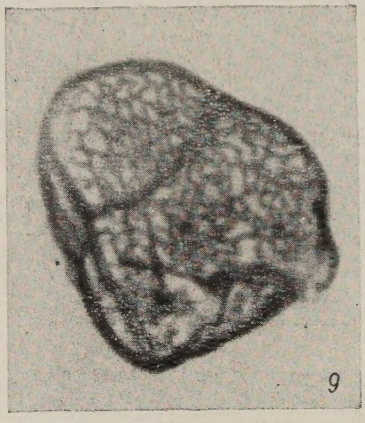


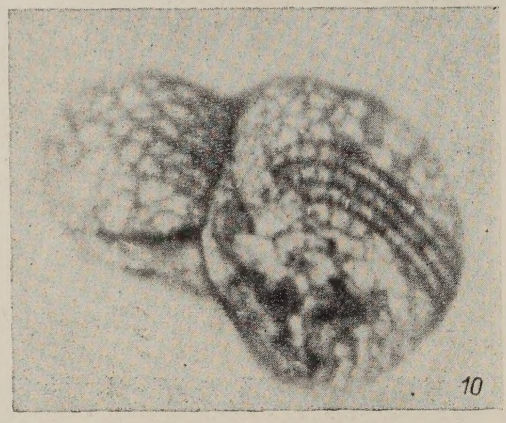


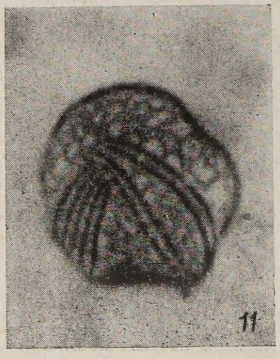


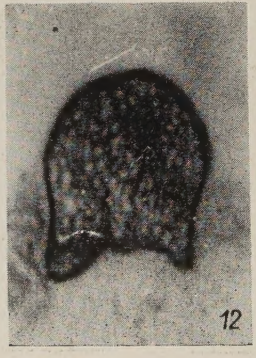


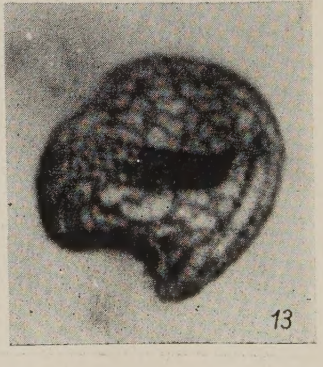


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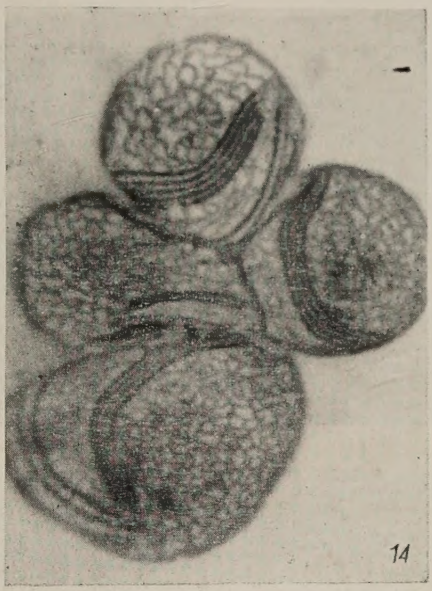
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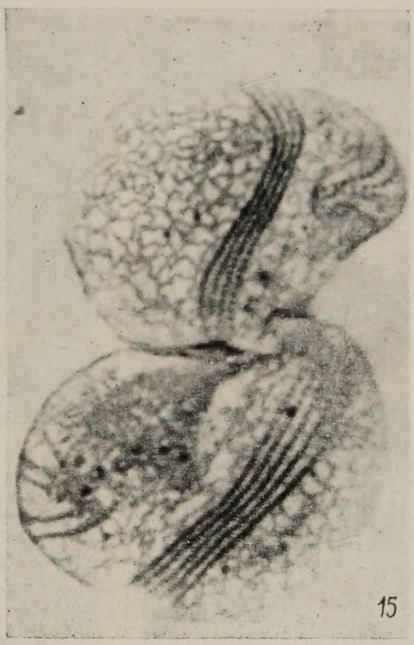
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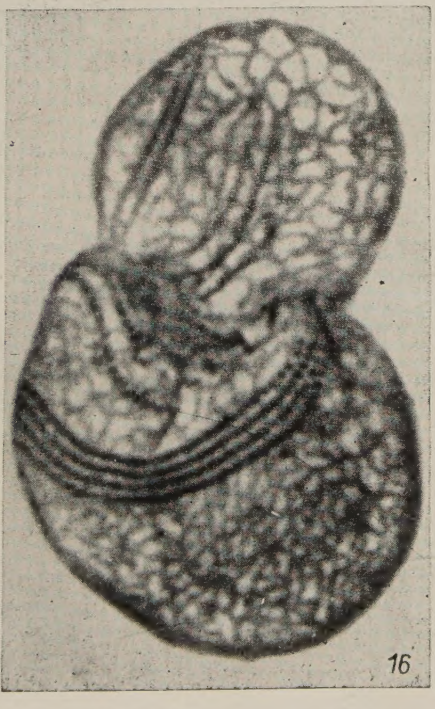
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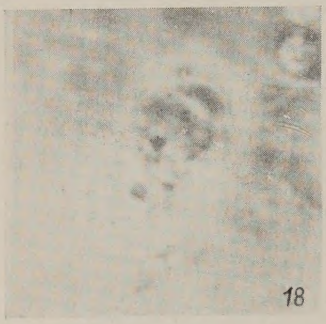


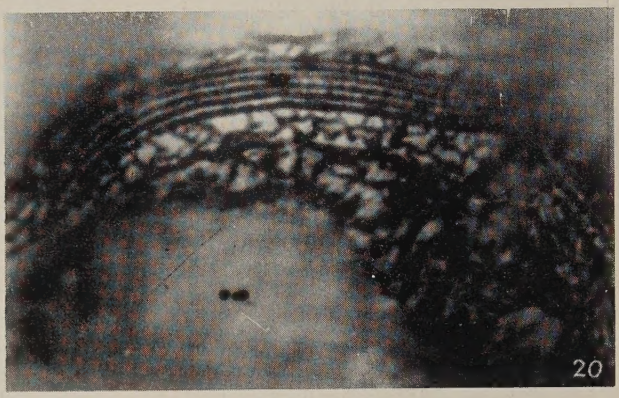


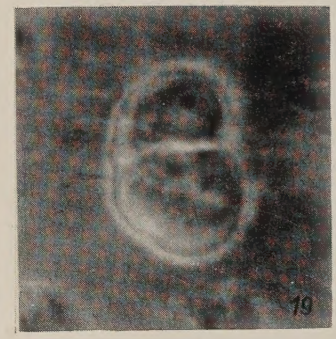


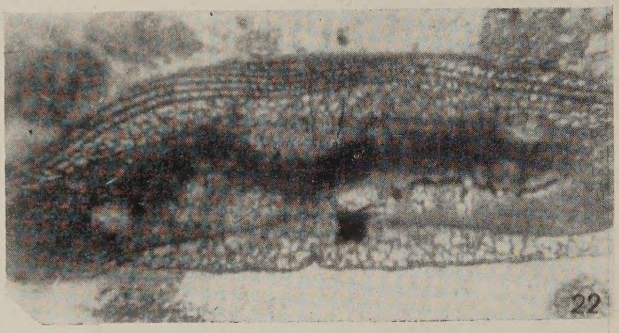


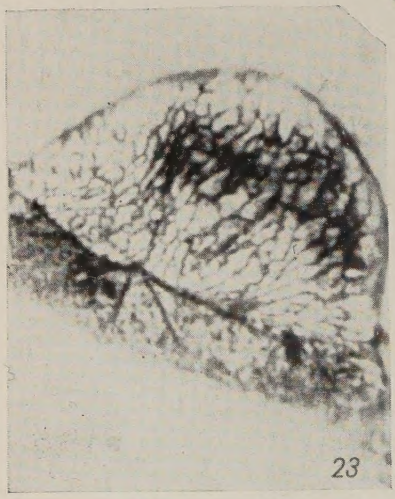

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